

PENDING CLAIMS AS A PRELIMINARY AMENDMENT

1. Method for detecting and/or purifying substances selected from proteins, biomolecules, complexes of proteins or biomolecules, subunits thereof, cell components, cell organelles and cells comprising the steps;

(a) providing an expression environment containing one or more heterologous nuclear acids encoding one or more polypeptides and/or one or more subunits of a biomolecule complex, the polypeptides or subunits being fused to at least two different affinity tags, one of which consists of one or more IgG binding domains of Staphylococcus protein A,

(b) maintaining the expression environment under conditions that facilitate expression of the one or more polypeptides or subunits in a native form as fusion proteins with the affinity tags,

(c) detecting and/or purifying the one or more polypeptides or subunits by a combination of at least two different affinity purification steps each comprising binding the one or more polypeptides or subunits via one affinity tag to a support material capable of selectively binding one of the affinity tags and separating the one or more polypeptides or subunits from the support material after substances not bound to the support material have been removed.

2. Method for detecting and/or purifying biomolecule and/or protein complexes, comprising the steps:

(a) providing an expression environment containing one or more heterologous nuclear acids encoding at least two subunits of a biomolecule complex, each being fused to at least one of different affinity tags, one of which consists of one or more IgG binding domains of Staphylococcus protein A,

2. maintaining the expression environment under conditions that facilitate expression of the one or more subunits in a native form as fusion proteins with the affinity tags, and under conditions that allow the formation of a complex between the one or more subunits and possibly other components capable of complexing with the one or more subunits,

(c) detecting and/or purifying the complex by a combination of at least two different affinity purification steps each comprising binding the one or more subunits via one affinity tag to a support material capable of selectively binding one of the affinity tags and separating the complex from the support material after substances not bound to the support material have been removed.

3. Method according to claim 1 or 2, wherein between the one or more polypeptides or subunits and one or more of the affinity tags a specific proteolytic cleavage site is present in the fusion protein which facilitates the removal of one or more of the affinity tags.

4. Method according to claim 3, wherein the specific proteolytic cleavage site is an enzymatic cleavage site.

5. Method according to claim 4, wherein the specific proteolytic cleavage site is the cleavage site for TEV protease N1A.

6. Method according to claim 3, wherein the proteolytic cleavage site is used to cleave the polypeptide residues in step (c) from the binding domain of the affinity tag and to remove the support material.

7. Method according to claim 6, wherein the affinity purification of step (i) comprises:

(i) binding the one or more polypeptides or subunits via the one or more IgG binding domains of Staphylococcus to a support material capable of specifically binding the latter, removing substances not bound to the support material and separating the one or more polypeptides or subunits from the support material by cleaving off the IgG binding domains via the specific proteolytic cleavage site, and

(ii) binding the polypeptides or subunit via another affinity tag to a second support material capable of specifically binding the latter, removing substances not bound to the support material and separating the polypeptide or subunit from the support material.

8. Method according to claim 7, wherein step (ii) is carried out before step (i).

9. Method according to claim 3, wherein the fusion protein contains a second specific proteolytic cleavage site for the removal of one or more of the other affinity tags.

10. Method according to claim 1 or 2, wherein one of the affinity tags consists of at least one calmodulin binding peptide.

11. Method according to claim 10, wherein a chemical agent is used to separate the one or more polypeptides or subunits from the support material.

12. Fusion protein comprising at least one polypeptide or subunit of a protein complex fused to at least two

different affinity tags, wherein one of the affinity tags consists of at least one IgG binding domain of Staphylococcus protein A.

13. Fusion protein according to claim 12, wherein it additionally contains a specific proteolytic cleavage site.

14. A nucleic acid coding for a fusion protein, the fusion protein comprising at least one polypeptide or subunit of a protein complex fused to at least two different affinity tags, wherein one of the affinity tags consists of at least one IgG binding domain of Staphylococcus protein A.

15. A vector comprising a nucleic acid under the control of sequences facilitating the expression of a fusion protein, the fusion protein comprising at least one polypeptide or subunit of a protein complex fused to at least two different affinity tags, wherein one of the affinity tags consists of at least one IgG binding domain of Staphylococcus protein A.

16. Vector comprising heterologous nucleic acid sequences in form of one or more cassettes each comprising at least two different affinity tags one consisting of one or more IgG binding domains of Staphylococcus aureus protein A, and at least one polynucleotide linker for the insertion of further nucleic acids.

17. Vector comprising heterologous nucleic acid sequences in form of two or more cassettes each comprising at least one of different affinity tags one consisting of one or more IgG binding domains of Staphylococcus aureus protein A, and at least one polynucleotide linker for the insertion of

Further limitations follow:

18. A cell containing a nucleic acid coding for a fusion protein or a vector comprising a nucleic acid under the control of sequences facilitating the expression of a fusion protein, the fusion protein comprising at least one polypeptide or subunit of a protein complex fused to at least two different affinity tags, wherein one of the affinity tags consists of at least one IgG binding domain of Staphylococcus protein A.

19. A reagent kit comprising:

a nucleic acid coding for a fusion protein or a vector for the expression of a fusion protein, wherein the fusion protein comprising at least one polypeptide or subunit of a protein complex fused to at least two different affinity tags, wherein one of the affinity tags consists of at least one IgG binding domain of Staphylococcus protein A; and

support materials each capable of binding at least one affinity tag.

20. Reagent kit according to claim 19 additionally comprising at least one chemical agent for separating one of the affinity tags from its support material and/or a specific chemical proteolytic agent and/or specific protease capable of cleaving the fusion protein.

21. A nucleic acid according to claim 14 or 15 wherein the fusion protein further comprises a specific proteolytic cleavage site.

22. The reagent kit of claim 19 wherein the vector

includes a nucleic acid under the control of a promoter facilitating the expression of fusion proteins.

25. The reagent kit of claim 19 wherein the vector comprising heterologous nucleic acid sequences in form of one or more cassettes each comprising at least two different affinity tags one consisting of one or more IgG binding domains of *Staphylococcus aureus* protein A, and at least one polynucleotide linker for the insertion of further nucleic acids.

26. The reagent kit of claim 19 wherein the vector comprises heterologous heterologous nucleic acid sequences in form of two or more cassettes each comprising at least one of different affinity tags one consisting of one or more IgG binding domains of *Staphylococcus aureus* protein A, and at least one polynucleotide linker for the further insertion of further nucleic acids.

27. A method for detection and/or purification of substances capable of complexing with fusion proteins, the method comprising contacting the fusion proteins with a sample and detecting and/or purifying substances capable of complexing with the fusion protein.

28. A method for detection and/or purification of cells and/or cell organelles expressing a fusion protein on their surface, the method comprising contacting the cells and/or cell organelles expressing a fusion protein on their surface with a substance capable of binding with the fusion protein, and detecting and/or purifying the cell and/or cell organelles expressing the fusion protein.